



Europäisches
Patentamt

European
Patent Office

Office européen
des brevets

REC'D 29 JUL 2003

WIPO

PCT

Bescheinigung

Certificate

Attestation

Die angehefteten Unterla-
gen stimmen mit der
ursprünglich eingereichten
Fassung der auf dem näch-
sten Blatt bezeichneten
europäischen Patentanmel-
dung überein.

The attached documents
are exact copies of the
European patent application
described on the following
page, as originally filed.

Les documents fixés à
cette attestation sont
conformes à la version
initialement déposée de
la demande de brevet
européen spécifiée à la
page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

02012302.2

**PRIORITY
DOCUMENT**
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

BEST AVAILABLE COPY



Anmeldung Nr:
Application no.: 02012302.2
Demande no:

Anmeldetag:
Date of filing: 04.06.02
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

BIOMAY Produktions- und Handels-
Aktiengesellschaft
Dr. Bohr-Gasse 7b
1030 Wien
AUTRICHE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se référer à la description.)

Allergen from mugwort pollen

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s)
revendiquée(s)

Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

C07K14/00

Am Anmeldetag benannte Vertragsstaaten/Contracting states designated at date of
filing/Etats contractants désignées lors du dépôt:

AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR

EPO - Munich
67
04. Juni 2002

June 4, 2002
K/Ka/Me

BIOMAY Produktions- und Handels-Aktiengesellschaft
Dr. Bohr-Gasse 7b
A-1030 Wien

Allergen from mugwort pollen

Pollen grains originating from weeds, in particular those belonging to the very extensive plant family *Compositae* or *Asteraceae*, are common allergen sources throughout the world. The three most important wind-pollinated genera in this family are *Ambrosia* (ragweed), *Parthenium* (feverfew), and *Artemisia vulgaris* (mugwort). In late summer and autumn, pollen of mugwort is one of the main causes of allergic reactions in central Europe and parts of Asia (J. Charpin, R. Surinyach, and A. W. Frankland (1974) Atlas of European Allergenic Pollens (Paris: Sandoz)). Approximately 10% of patients suffering from pollinosis are sensitized by mugwort pollen. Ragweed pollen represents the major source of allergenic proteins in the United States and Canada, with a prevalence of about 50% in atopic individuals (L. P. Boulet, H. Turcotte, C. Laprise, C. Lavertu, P. M. Bedard, A. Lavoie, and J. Hebert (1997) Comparative degree and type of sensitization to common indoor and outdoor allergens in subjects with allergic rhinitis and/or asthma. Clin Exp Allergy 27, 52). In Europe, ragweed allergy is not a major cause of pollinosis, but it is rapidly increasing. Ragweed was first introduced into Europe at the beginning of last century and was limited to Hungary. During the Second World War it was introduced in France, thereafter gradually expanded and is now abundant in the Rhone valley (France), northern Italy, eastern parts of Austria, Hungary and Bulgaria (G. D'Amato, F. T.

Spieksma, G. Liccardi, S. Jager, M. Russo, K. Kontou-Fili, H. Nikkels, B. Wuthrich, and S. Bonini (1998) Pollen-related allergy in Europe. *Allergy* 53, 567).

Nowadays, it is widely accepted that recombinant allergens represent promising tools for diagnosis and therapy of Type I allergy. The value of these molecules for diagnosis has been evaluated in detail and a panel of recombinant allergens is already available for routine diagnosis of certain inhalant allergies, including tree (birch)- and grass- pollen allergies (R. Valenta et al. (1992) *Int Arch Allergy Immunol* 97, 287; O. Scheiner, and D. Kraft (1995) *Allergy* 50, 384; D. Kraft et al. (1998) *Allergy* 53, 62; D. Kraft et al. (1999) *Int Arch Allergy Immunol* 118, 171) but not for weed pollen-allergy. Two major allergenic compounds of ragweed pollen known as antigens E (Amb a 1) and K (Amb a 2) were characterized and shown to be acidic proteins of about 38,000 Da (T. P. King (1980) *Allergy* 35, 187). cDNA cloning of Amb a 1 and Amb a 2 revealed that these allergens are highly homologous to each other and belong to the family of pectate lyase proteins (T. Rafnar et al. (1991) *J Biol Chem* 266, 1229; B. L. Rogers et al. (1991) *J Immunol* 147, 2547). Presently, no information about the complete structure and no molecular cloning data of any mugwort pollen allergen have been published. The only exception is a mugwort pollen allergen designated Art v 1 which is disclosed in WO99/49045. Art v 1 is not related to Amb a 1 or to any other characterized ragweed allergen. Several reports demonstrated that ragweed and mugwort pollen share common allergenic structures, i.e. antigens recognized by cross-reactive IgE antibodies in different allergen sources (C. Fernandez et al. (1993) *J Allergy Clin Immunol* 92, 660; R. Hirschehr et al. (1998) *J Allergy Clin Immunol* 101, 196; H. S. Park et al. (1994) *J Korean Med Sci* 9, 213). IgE recognition of such common antigens will trigger allergic reactions in sensitized patients after contact with different allergen sources. Therefore, the characterization of mugwort pollen allergens and identification of common allergenic structures in ragweed pollen will provide basic information for the selection and production of recombinant allergens that are sufficient for the diagnosis and therapy of weed pollen-allergy.

It is therefore an object of the present invention to identify an allergen from mugwort pollen which might trigger type I allergy.

The inventors succeeded in isolating four *bona fide* and complete cDNA clones corresponding to a 4.8 kDa mugwort (*Artemisia vulgaris*) pollen allergen. The amino acid

The invention therefore relates to a polypeptide comprising an amino acid sequence which has an identity of at least 70% to the amino acid sequence as shown in SEQ ID NO:1. Preferably the polypeptide comprises an amino acid sequence which has an identity of at least 80%, more preferably an identity of at least 90%, most preferably an identity of at least 95% to the amino acid sequence as shown in SEQ ID NO:1. In a particular embodiment, the polypeptide comprises the amino acid sequence as shown in SEQ ID NO:1. As used herein, the term polypeptide refers to proteins and/or peptides having a length of at least 7 amino acids.

The degree of identity of an amino acid sequence to SEQ ID NO:1 is determined by comparing said amino acid sequence and SEQ ID NO:1 using the program "BLAST 2 SEQUENCES (blastp)" (Tatusova et al. (1999) FEMS Microbiol Lett. 174, 247-250) with the following parameters: Matrix BLOSUM62; Open gap 11 and extension gap 1 penalties; gap x_dropoff 50; expect 10.0 word size 3; Filter: none. According to the invention the sequence comparison covers at least 100 amino acids, preferably at least 200 amino acids, more preferably at least 300 amino acids, most preferably at least 350 amino acids.

The invention further relates to a polypeptide comprising a fragment of at least 18 amino acids of the amino acid sequence as shown in SEQ ID NO:1. Such polypeptide comprises at least 18 consecutive amino acids of the sequence as shown in SEQ ID NO:1. Preferably, the length of the fragment is at least 21 amino acids, more preferably at least 25 amino acids, even more preferably at least 35 amino acids, most preferably at least 50 amino acids of the amino acid sequence as shown in SEQ ID NO:1.

Another aspect of the invention is a polypeptide consisting of a fragment of at least 7 amino acids of the amino acid sequence as shown in SEQ ID NO:1. Preferably, the length of said polypeptide is at least 10 amino acids, more preferably at least 15 amino acids, even more preferably at least 25 amino acids, most preferably at least 35 amino acids. Polypeptides consisting of at least 7 amino acids can be recognized by T cells (T cell epitopes).

Yet another aspect of the invention is a polypeptide comprising a fragment of the amino acid sequence as shown in SEQ ID NO:1 wherein said fragment is capable of binding to IgE antibodies from an individual being allergic against mugwort pollen. The term "IgE

antibodies" means an antibody preparation which is obtainable by per se known processes. In susceptible humans, exposure to allergens leads to an immediate type (IgE-mediated) of allergic response, which is a two-step process:

(1) On the first exposure, allergenic proteins induce IgE synthesis by B-cells (Vercelli and Geha, 1989, J. Allergy Clin. Immunol. 9: 75-83). These specific IgE antibodies then bind to the surfaces of mast cells and basophils via high-affinity Fcε receptors (FcεRI).

(2) On subsequent exposure, allergens bind and crosslink these specific IgE antibodies leading to the release of pre-formed and newly synthesized inflammatory mediators (e.g. histamine) and chemotactic substances (e.g. platelet-activating factor).

In step (1), the production of allergen-specific IgE by B lymphocytes requires the "help" of T lymphocytes (Vercelli and Geha, 1989, J. Allergy Clin. Immunol. 9: 75-83), which are activated by linear peptide fragments of the allergen. These peptides are created by antigen-presenting cells through antigen processing and are displayed at the cell surface with molecules of the major histocompatibility complex (MHC), where they become available for recognition and binding to the T cell receptor (Schwartz, 1985, Annu Rev. Immunol. 3: 237-255; Rothbart et al., 1989, Int. Immunol. 1: 479-486). In step (2), allergen-specific IgE antibodies produced by B cells circulate and bind to FcεRI receptors on mast cells and basophils, thereby serving as the receptor for the allergen. Cross-linking of these cell surface-bound IgE antibodies by allergen represents the signal for the release of preformed and newly synthesized inflammatory mediators and chemotactic substances, leading to the typical allergic inflammatory reaction.

An individual being allergic against mugwort pollen is an individual that displays specific IgE antibodies recognizing mugwort pollen proteins and have typical allergic symptoms (IgE-mediated) when exposed to mugwort pollen. Such allergic reactions can also be triggered by exposure to homologous antigens present in other allergen sources. This phenomenon is defined as cross-reactivity. The selection of patients allergic to mugwort pollen was done according to typical case history, positive skin prick test and radioallergosorbent test "RAST" >3.5.

A polypeptide is capable of binding to an antibody if its affinity to the antibody is significantly higher than that of a reference composition which does not bind to the antibody. The binding of specific IgE antibodies to a particular polypeptide or allergen can be determined by various methods, e.g. RAST, Immunoblot ELISA, using as reference

sera from healthy non-allergic individuals (according to case history, negative skin prick test, negative RAST), who do not display allergen-specific IgE antibodies.

In one embodiment of the invention the polypeptide is capable of binding to IgE antibodies from an individual being allergic against ragweed pollen. The present invention makes it possible to identify allergenic structures which are common to mugwort pollen and ragweed pollen. This information is useful for the selection and production of recombinant allergens that are sufficient for the diagnosis and therapy of weed pollen-allergy. Peptides and polypeptides containing such common allergenic structures are encompassed by the present invention.

During purification of natural Amb a 1 trypsin-like pollen protease cleaves this allergen. Thus, purification often results in the isolation of a full-length 38 kDa allergen plus two fragments of 26 and 12 kDa molecular weight, designated alpha (amino acids 187 to 396, numbering including signal peptide) and beta (amino acids 43 to 180, numbering including signal peptide), respectively. All chains are still highly reactive with polyclonal rabbit antibodies and human IgE from allergic patients. It is expected that proteolytic fragments of the natural 40.9 kDa mugwort allergen corresponding to these chains would also bind IgE antibodies from allergic patients. The first one corresponding to beta chain spans amino acids 21 to 180 and the second one corresponding to alpha chain spans amino acids 181 to 396, both numberings referring to SEQ ID NO:2. Both chains are expected to have epitopes which are also present in the Amb a 1 chains resulting in the same IgE reactivity.

According to a preferred embodiment, the polypeptides of the invention are isolated polypeptides, i.e. they are in an essentially pure form. "Essentially pure" means that by separation of the polypeptides from other compounds the polypeptides are at least 75% pure, preferably at least 90 % pure, more preferably at least 95% pure. The purity of polypeptides can be determined by the skilled person using techniques known in the field, e.g. by SDS-PAGE followed by protein staining. High Performance Liquid Chromatography (HPLC) and mass spectrometry are methods suitable for analyzing polypeptides and short peptides as well.

The polypeptides of the invention can be prepared in various ways. The polypeptides may be prepared by chemical synthesis, preferably by applying solid phase methods. Methods of chemical peptide synthesis are known to the skilled person. These methods are

particularly suited for the preparation of short polypeptides having a length of 7 to 50 amino acids. Longer polypeptides may be prepared by chemically or enzymatically linking peptide fragments which have been prepared by chemical synthesis. The polypeptides of the invention may also be prepared by expression of DNA sequences in host cells. These methods are described in more detail below.

The present invention also concerns various polynucleotides. The polynucleotides may be single or double stranded DNA molecules, RNA molecules or nucleic acids which can be derived therefrom. According to a first aspect, the polynucleotides of the invention encode the amino acid sequence as shown in SEQ ID NO:1. Due to the degeneracy of the genetic code, many different polynucleotides can be envisaged which encode the amino acid sequence as shown in SEQ ID NO:1.

According to a second aspect, the polynucleotides of the invention encode a polypeptide according to the invention as described supra.

The polynucleotides described herein can be used to identify similar sequences in any variety or type of mugwort and, thus, to identify or isolate sequences which have sufficient homology to hybridize to, for example, DNA from mugwort pollen. This can be carried out, for example, under conditions of low stringency; those sequences which have sufficient homology (generally greater than 40%) can be selected for further assessment. Alternatively, high stringency conditions can be used.

In this manner, DNA of the present invention can be used to identify, in other types of weed pollen sequences encoding peptides having amino acid sequences similar to that of the 40.9 kDa protein described herein and, thus, to identify allergens in such other types of weed pollen. Thus, the present invention includes not only the 40.9 kDa protein and other mugwort allergens encoded by the present DNA sequences, but also other weed pollen allergens encoded by DNA which hybridizes to DNA of the present invention.

Therefore, according to a third aspect, the polynucleotides of the invention comprise a nucleotide sequence which has an identity of at least 75 % to the nucleotide sequence as shown in SEQ ID NO:2. Preferably, the identity is at least 80 %, more preferably at least 90 %, most preferably at least 95%. The degree of identity of a polynucleotide sequence to SEQ ID NO:2 is determined by comparing said polynucleotide sequence and SEQ ID NO:2

Microbiol Lett. 174, 247-250) with the following parameters: Reward for a match 1; Penalty for a mismatch -2; Open gap 5 and extension gap 2 penalties; gap x_dropoff 50; expect 10.0; word size 11; Filter: None. According to the invention the sequence comparison covers at least 300 nucleotides, preferably at least 600 nucleotides, more preferably at least 900 nucleotides, most preferably at least 1100 nucleotides.

In one embodiment the polynucleotide comprises the nucleotide sequence as shown in SEQ ID NO:2 or the nucleotide sequence as shown in SEQ ID NO:3.

The invention also covers polynucleotides which are degenerate to the polynucleotides described supra. The respective complementary strands of the polynucleotides disclosed are encompassed by the present invention.

The polynucleotides of the present invention preferably are isolated polynucleotides. The polynucleotides preferably are essentially pure, i. e. they are at least 80 % pure, more preferably at least 90 % pure, most preferably at least 95 % pure. The polynucleotides according to the invention can be prepared in various ways. They may be synthesized by chemical methods usually employed in oligonucleotide synthesis. PCR based methods can be used to synthesize the polynucleotides, in particular longer polynucleotides. Longer polynucleotides can also be prepared by chemically or enzymatically linking fragments which have been synthesized using chemical methods.

Another aspect of the invention is a plasmid or a vector comprising a polynucleotide according to the invention. The plasmids may contain regulatory sequences which facilitate replication of the plasmids or transcription and/or translation of encoded sequences. Examples of such sequences are promoters, terminator sequences, etc. The plasmids may also contain nucleotide sequences encoding amino acid sequences which facilitate the purification of encoded polypeptides upon expression in a host cell. Examples of such sequences are a 6xHis tag, a FLAG tag, and sequences encoding bacterial proteins such as GST. Purification can be achieved by affinity chromatography using antibodies directed against the respective tag sequences or bacterial sequences. Plasmids and vectors containing such tag sequences or bacterial sequences are known to the skilled person.

The invention further pertains to a cell containing a plasmid or a vector and/or a polynucleotide according to the present invention. The cells may be selected from plant

cells, bacterial cells, yeast cells and other cells. Preferred are cells which allow for the expression of the genes encoded by the polynucleotides of the invention. Most preferably, the cells are *E. coli* cells. The plasmid, vector and/or polynucleotide according to the present invention may be introduced into the cells by techniques known per se, e.g. transformation, transfection etc. The cells can contain the nucleic acid molecules only transiently or stably integrated into their genome. In particular in the latter case, the nucleic acid molecules may be truncated or fragmented due to the process of integration into the genome. Cells containing such truncated or fragmented versions of the nucleic acid molecules are within the scope of the present invention.

The cells can be used for the expression and purification of the polypeptides of the invention. The invention therefore relates to a process for the preparation of a polypeptide according to the invention comprising the step of culturing the cells described in the present invention under conditions appropriate for the expression of the polypeptide and optionally subsequently recovering the polypeptide. The cells are preferably cultured in a liquid medium under agitation. If appropriate, gene expression is induced by an inducing agent, e.g. IPTG.

Following cultivation the cells may be opened using established methods and the polypeptide may be recovered by affinity chromatography. Other known methods of protein purification can be employed.

Polypeptides of the present invention can be used, for example, as "purified" allergens. Such purified allergens are useful in the standardization of allergen extracts which are key reagents for the diagnosis and treatment of ragweed allergy. Furthermore, by using peptides based on fragments of the amino acid sequence as shown in SEQ ID NO:1, anti-peptide antisera or monoclonal antibodies can be made using standard methods. These sera or monoclonal antibodies, directed against the 40.9 kDa protein of the invention, can be used to standardize allergen extracts.

Another aspect of the invention is an antibody which is capable of binding to a polypeptide according to the present invention. The antibody may be a polyclonal or a monoclonal antibody, monoclonal antibodies, however, are preferred. In a particular embodiment the antibody is in an essentially pure form, i.e. at least 80% pure, preferably at least 90% pure.

In the case of polyclonal antibodies, the antibody composition contains many different species of antibody molecules.

The antibody may be specific for the polypeptide comprising the amino acid sequence as shown in SEQ ID NO:1. In this embodiment, the antibody does not bind to anyone of the polypeptides Amb a 1.1, Amb a 1.2, Amb a 1.3 and Amb a 2 from ragweed pollen.

In another embodiment, however, the antibody is capable of binding to one or several of the polypeptides Amb a 1.1, Amb a 1.2, Amb a 1.3 and Amb a 2 from ragweed pollen.

Antibodies according to the invention can be used to isolate additional components of mugwort allergens which can be used for further definition of the characteristics of this family of allergens. Furthermore, anti-peptide sera or monoclonal antibodies directed against the polypeptides of the invention can be used to standardize and define the content of skin test reagents.

The invention further concerns a pharmaceutical composition comprising a polypeptide and/or a polynucleotide and/or an antibody according to the present invention.

The materials described herein, as well as compositions containing these materials, can be used in methods of diagnosing, treating and preventing mugwort allergy. Another aspect of the invention therefore is the use of a polypeptide and/or a polynucleotide and/or an antibody according to the present invention for the preparation of a medicament for the treatment or the prevention or the diagnosis of an allergic disorder.

The medicament is preferably administered to an individual to be desensitized.

Through use of the polypeptides of the present invention, allergen preparations of consistent, well-defined composition and biological activity can be made and administered for therapeutic purposes (e.g., to modify the allergic response of a mugwort-sensitive individual to a mugwort pollen). Such polypeptides or modified versions thereof may, for example, modify B-cell response to a mugwort allergen, T-cell response to a mugwort allergen or both responses. Purified allergens can also be used to design modified derivatives or analogues which are more useful in immunotherapy than are the unmodified naturally-occurring peptides.

High doses of allergens generally produce the best symptom relief. However, many people do not tolerate high doses of allergens because of allergic reactions to the allergens. Modification of naturally-occurring allergens can be designed in such a manner that modified polypeptides which have the same or enhanced therapeutic properties as the corresponding naturally-occurring allergen but have reduced side effects, especially reduced anaphylactic reactions, can be produced. These can be, for example, a polypeptide of the present invention or a modified analogue (e.g., a polypeptide in which the amino acid sequence has been altered to modify immunogenicity and/or reduce allergenicity or to which a component has been added for the same purpose). For example, the polypeptides can be modified using the polyethylene glycol method of A. Sehon and co-workers. Short segment deletion or amino acid substitutions of the polypeptide sequence results in a weakened (weaker or no) antibody binding. This modification leads to low IgE binding and is very useful in immunotherapy due to less side effects.

Administration of the peptides of the present invention to an individual to be desensitized can be carried out using known techniques. A peptide or combination of different peptides can be administered to an individual in a composition which includes, for example, an appropriate buffer, a carrier and/or an adjuvant. Such compositions will generally be administered by injection, oral administration, inhalation, transdermal application or rectal administration. Using the structural information now available, it is possible to design a mugwort pollen polypeptide which, when administered to a mugwort-sensitive individual in sufficient quantities, will modify the individual's allergic response to a mugwort allergen. This can be done, for example, by examining the structures of the mugwort proteins, producing peptides to be examined for their ability to influence B-cell and/or T-cell responses in mugwort-sensitive individuals and selecting appropriate epitopes recognized by the cells. Synthetic amino acid sequences which mimic those of the epitopes and which are capable of down regulating allergic response to mugwort allergen can also be used.

Polypeptides or antibodies of the present invention can also be used for detecting and diagnosing mugwort allergy. For example, by combining blood or blood products obtained from an individual to be assessed for sensitivity to mugwort allergen with an isolated allergenic peptide of mugwort pollen, under conditions appropriate for binding of

components (e.g., antibodies, T cells, B cells) in the blood with the polypeptide and determining the extent to which such binding occurs.

It is now also possible to design an agent or a drug capable of blocking or inhibiting the ability of mugwort allergens to induce an allergic reaction in mugwort-sensitive individuals. Such agents could be designed, for example, in such a manner that they would bind to relevant anti-mugwort IgEs, thus preventing IgE-allergen binding and subsequent mast cell degranulation. Alternatively, such agents could bind to cellular components of the immune system, resulting in suppression or desensitization of the allergic response to mugwort allergens. A non-restrictive example of this is the use of appropriate B- and T-cell epitope peptides, or modifications thereof, based on the cDNA/polypeptide structures of the present invention to suppress the allergic response to mugwort allergens. This can be carried out by defining the structures of B- and T-cell epitope peptides which affect B- and T-cell function in in vitro studies with blood cells from mugwort-sensitive individuals.

Finally, the invention relates to kits which are useful for the diagnosis, the treatment and/or the prevention of an allergic disorder comprising a polypeptide and/or a polynucleotide and/or an antibody according to the present invention. The materials described herein, as well as compositions and kits containing these materials, can be used in methods of diagnosing, treating and preventing mugwort allergy.

The present invention is based on a recombinant DNA molecule and fragments thereof coding for a 40.9 kDa mugwort (*Artemisia vulgaris*) pollen allergen, which shows sequence homology to Amb a 1, a major allergen of ragweed (*Ambrosia artemisiifolia*) pollen, on a method for the isolation of Art v 1 molecules or fragments thereof, and on an expression vector and a host cell transformed to express the nucleic acid sequences of the invention. The present invention further relates to methods of diagnostics and therapy of weed pollen-allergic patients. Because the 40.9 kDa mugwort allergen shows sequence homology to Amb a 1, this mugwort protein might represent a cross-reactive allergen and could be used for diagnosis and therapy of not only mugwort pollen allergy, but of weed pollen-allergy in general.

The present application describes the isolation of four *bona fide* and complete cDNA clones corresponding to a 40.9 kDa mugwort (*Artemisia vulgaris*) pollen allergen. The cDNA nucleotide sequences and derived amino acid sequences of the clones M4, M6, and

M15 were identical (See Figures 1 and 2). Clone M8 differed from the other by 8 nucleotide substitutions, which did not lead to amino acid exchanges (See Figure 3). The clones are complete in their 5' ends because they contain the start AUG codon in a typical eukaryotic context and some sequence upstream of the start codon. The clones are complete in the 3' region because they contain 90-120 nucleotides after the stop codon followed by the polyA⁺-tail. The sequences outside the open reading frame are not shown in Figures 2 and 3. The alignments of the nucleotide sequences of clones M4, M6, M8 and M15 are shown in Figure 1A-D. The nucleotide sequence of the coding region and the deduced amino acid sequence of clones M4, M6 and M15 are shown in Figure 2. The nucleotide sequence of the coding region and the deduced amino acid sequence of clone M8 are shown in Figure 3.

Figures 1A, 1B, 1C and 1D show an alignment of the nucleotide sequences of clones M4, M6, M8 and M15 (SEQ ID NO:6, 7, 8, and 9). Capitals in the consensus sequence indicate identical nucleotides in the four clones.

Figure 2 shows the nucleotide sequence of the coding region (SEQ ID NO:2 plus stop codon) and the deduced amino acid sequence (SEQ ID NO:1) of clones M4, M6 and M15.

Figure 3 shows the nucleotide sequence of the coding region (SEQ ID NO:3 plus stop codon) and the deduced amino acid sequence (SEQ ID NO:1) of clone M8.

Figure 4 shows the structure of the multiple cloning site of plasmid "pHis-Parallel2" as used in Example 2. The amino acids encoding a 6xHis tag, a spacer region and a protease cleavage site are also indicated.

Figure 5A shows Mugwort pollen extract, purified mugwort pollen allergen and purified Amb a 1 from ragweed pollen separated by gel electrophoresis followed by Coomassie staining. Figure 5B shows an immunoblot with rabbit anti-Amb a 1 antibodies (See Examples 3 and 4).

Figure 6 shows an immunoblot with IgE antibodies (See Examples 3 and 4).

The present invention will be further illustrated by the following examples which are not intended to be limiting.

Example 1: Immunoscreening of a Mugwort Pollen cDNA Library with Purified Rabbit anti-Amb a 1 Antibodies

The isolation of the cDNA clone coding for the 40.9 kDa mugwort pollen allergen was done in the following way:

The first step for the cDNA cloning of the 40.9 kDa allergen was the isolation of RNA from mugwort pollen, which turned out to be a very difficult procedure. Using several different standard procedures for RNA isolation, there were always pigments and other organic substances co-purifying with the RNA that inhibited enzymes used for the cDNA synthesis. However, after a series of trials it was possible to isolate mRNA sufficiently pure to construct a cDNA library, which was also done in the expression vector lambda ZAP.

Sera from rabbits immunized with Amb a 1 was kindly provided by Dr. Te Piao King, (Rockefeller University, USA). For screening the mugwort cDNA library we first purified Amb a 1-specific antibodies by affinity chromatography. For that purpose, 5 mg of Amb a 1 purified from ragweed pollen was coupled to CNBr-activated Sepharose (Pharmacia). After binding of the rabbit sera, the resin was washed and Amb a 1-specific antibodies eluted with 0.2 M glycine, pH 2.8. The antibodies were neutralized and dialyzed against PBS. With these purified rabbit anti-Amb a 1-antibodies 450.000 plaques of a mugwort pollen cDNA library constructed in lambda ZAP II phages (Stratagene, CA, USA) were screened. In total, 13 positive clones were isolated and used for single clone *in vivo excision* of pBluescript phagemid from the Uni- ZAP XR vector. Four clones (M4, M6, M8, and M15) were selected for DNA sequence analysis, which was carried out by the "primer walking" technique using 5 primers including the flanking primers T7 and T3. Both strands were sequenced twice. The aligned nucleotide sequences of the four clones M4, M6, M8, and M15 are shown in Figures 1A-1D. The cDNA sequences of clones M4, M6 and M15 were identical (Figure 2). The cDNA sequence in the coding region of M8 differed from the other 3 clones by 8 bases (Figure 3). However, all four clones lead to the same protein with a calculated molecular weight of 40.9 kDa (Figures 2 and 3).

Finally, the sequence of the 40.9 kDa protein was used for similarity searches in the Database. Significant sequence homology was found to Amb a 1, a major allergen from ragweed pollen belonging to the pectate lyase family (63.9 % identity).

The cDNA of the invention can now be obtained based on the sequence information as described herein. The cDNA can be cloned from a cDNA library using as a probe an oligonucleotide or polynucleotide which was prepared by chemical synthesis. Alternatively, the cDNA can be obtained by PCR using primers which are derived from SEQ ID NO:2. Such methods are known to the skilled person.

Example 2: Cloning of the 40.9 kDa mugwort allergen (clone M4) into the expression plasmid pHis- parallel-2

The expression plasmid containing the 40.9 kDa mugwort allergen cDNA was constructed in the vector pHis-parallel 2 (see Figure 4). For the cloning procedure two flanking cloning primers were constructed. The complete cDNA sequence was truncated at the 5' end by 60 nucleotides coding for the putative signal peptide. In this way only the mature form of the protein was produced in *E. coli*.

The following primers were used: Mug-Nco-fwd 5' GAG AGA GAC CAT GGC TCG GGC TGA CAT TGG TGA TGA GCT CG 3' (SEQ ID NO:4) and Mug-Xho-rev 5' GAG AGA GAC TCG AGT TAA CAA GGT TTT CCA GGA ACG CAT TTG 3' (SEQ ID NO:5) for PCR, Nco I and Xho I restriction sites were introduced at the 5' and 3' ends. PCR products were digested with Nco I and Xho I restriction enzymes and ligated to the respective sites of the vector pHis-parallel-2. The clone was analyzed by DNA sequencing.

Example 3: Expression of the recombinant 40.9 kDa mugwort allergen in *E. coli*

For the recombinant production of the 40.9 kDa mugwort allergen, competent *E. coli* strain BL21 (DE3, Stratagene, CA, USA) was transformed with the expression plasmid pHis-parallel-2-M4 and selected on LB plates containing 100 mg/l ampicillin. A single transformant colony was picked and bacteria were grown in a 25 ml overnight pre-culture with LB medium containing ampicillin to an optical density of 0.6 at 600 nm. LB-amp

added to a final concentration of 1 mM and incubation continued for 7 hours at 37°C. The bacterial cells were harvested by centrifugation and pellets were resuspended in 50 mM phosphate buffer pH8 containing 300 mM NaCl and 10 mM imidazole. Cells were then disrupted by three cycles of freezing in liquid nitrogen, followed by thawing at 37°C. After centrifugation at 5,500 rpm for 25 minutes, the supernatant was removed (soluble fraction) and 6 M urea was added to the pellet. After centrifugation, the supernatant was removed (insoluble fraction), and both soluble and insoluble fractions analyzed by SDS-PAGE. The recombinant 40.9 kDa mugwort protein was recovered in the insoluble fraction, which was then used for immunoblots with anti-Amb a 1 antibodies (Figure 5B, lane 4) and with serum IgE from weed pollen-allergic patients (Figure 6, lanes 3 and 4). As control, *E. coli* strain BL21 transformed with pHis-parallel-2 without an insert was processed as described above and the insoluble fraction used for the immunoblot experiments (Figure 6, lanes 5 and 6).

Figure 5A, lane 1 shows the mugwort pollen extract which contains all the proteins (allergens or nonallergens) released from the pollen.

Preparation of the mugwort pollen extract:

150 mg of mugwort pollen grains (Allergon AB, Sweden) were mixed with 1 ml of water. The pollen suspension was gently agitated at room temperature for a 10 hour period. After centrifugation the pattern of proteins released from the mugwort pollen was analyzed by SDS-PAGE and Coomassie staining.

Lane 2 of Figure 5A shows purified natural 40.9 kDa mugwort pollen allergen. The purification was done via immunoaffinity chromatography method. Purified rabbit anti Amb a 1 antibodies were coupled to a cyanogen bromide activated sepharose CL4B (Pharmacia Biotech) and used for the purification of the natural 40.9 kDa mugwort protein from a filtered 10 hours pollen extract.

Lane 3 of Figure 5A shows purified natural Amb a 1, a major allergen in the ragweed pollen. The purification was also done via immunoaffinity chromatography method. Purified rabbit anti Amb a 1 antibodies were coupled to a cyanogen bromide activated sepharose CL4B (Pharmacia Biotech) and used for the purification of the natural Amb a 1 from a filtered 10 hours ragweed pollen extract including 10 mM protease inhibitor 1,10 phenantroline.

Example 4: Purification of the natural 40.9 kDa allergen from mugwort pollen

The natural 40.9 kDa allergen was purified from aqueous extract of mugwort pollen by immunoaffinity chromatography. Purified rabbit anti-Amb a 1 antibodies were coupled to CNBr-activated sepharose CL4B and used for the purification of the 40.9 kDa allergen. The purified natural 40.9 kDa allergen was then tested in immunoblots with anti-Amb a 1 antibodies (Figure 5A, lane 2) and with IgE antibodies from allergic patients (Figure 6, lanes 1 and 2).

A cross-reactivity is occurring between the US patient sensitized for ragweed and the purified natural and recombinant 40.9 kDa protein in mugwort. Therefore a positive signal can be observed in Figure 6A and 6B. It is expected that many more patients show a cross-reactivity between these two allergens.

Claims

1. A polypeptide selected from the group consisting of:
 - a) polypeptides comprising a fragment of at least 18 amino acids of the amino acid sequence as shown in SEQ ID NO:1;
 - b) polypeptides comprising an amino acid sequence which has an identity of at least 70% to the amino acid sequence as shown in SEQ ID NO:1;
 - c) polypeptides comprising a fragment of the amino acid sequence as shown in SEQ ID NO:1 wherein said fragment is capable of binding to IgE antibodies from an individual being allergic against mugwort pollen; and
 - d) polypeptides consisting of a fragment of at least 7 amino acids of the amino acid sequence as shown in SEQ ID NO:1.
2. A polypeptide according to claim 1 comprising the amino acid sequence as shown in SEQ ID NO:1.
3. A polypeptide according to claim 1 or 2 characterized in that it is capable of binding to IgE antibodies from an individual being allergic against ragweed pollen.
4. A polynucleotide selected from the group consisting of
 - a) polynucleotides encoding the amino acid sequence as shown in SEQ ID NO:1;
 - b) polynucleotides encoding a polypeptide as claimed in claim 1; and
 - c) polynucleotides comprising a nucleotide sequence which has an identity of at least 75 % to the nucleotide sequence as shown in SEQ ID NO:2;

or the complementary strand of such polynucleotide.

5. A polynucleotide according to claim 4 comprising the nucleotide sequence as shown in SEQ ID NO:2 or the nucleotide sequence as shown in SEQ ID NO:3.
6. A plasmid or a vector comprising a polynucleotide as claimed in claim 4 or 5.
7. A cell containing a plasmid or a vector as claimed in claim 6 and/or a polynucleotide as claimed in claim 4 or 5.
8. A cell according to claim 7 which is selected from the group consisting of plant cells, bacterial cells and yeast cells.
9. A process for the preparation of a polypeptide as claimed in anyone of claims 1 to 3 comprising the step of culturing cells as claimed in claim 7 or 8 under conditions appropriate for the expression of the polypeptide and optionally subsequently recovering the polypeptide.
10. A process according to claim 9 wherein the cells are opened and the polypeptide is recovered using affinity chromatography.
11. An antibody capable of binding to a polypeptide as claimed in anyone of claims 1 to 3.
12. An antibody according to claim 11 which is capable of binding to one or several of the polypeptides selected from the group consisting of Amb a I.1, Amb a I.2, Amb a I.3 and Amb a 2.
13. An antibody according to claim 11 which does not bind to anyone of the polypeptides selected from the group consisting of Amb a I.1, Amb a I.2, Amb a I.3 and Amb a 2.
14. A pharmaceutical composition comprising a polypeptide as claimed in anyone of claims 1 to 3 and/or a polynucleotide as claimed in claim 4 or 5 and/or an antibody as claimed in anyone of claims 11 to 13.

15. The use of a polypeptide as claimed in anyone of claims 1 to 3 or a polynucleotide as claimed in claim 4 or 5 or an antibody as claimed in anyone of claims 11 to 13 for the preparation of a medicament for the treatment or the prevention or the diagnosis of an allergic disorder.

16. A use according to claim 15 wherein the medicament is administered to an individual to be desensitized.

17. A kit useful for the diagnosis, the treatment and/or the prevention of an allergic disorder comprising a polypeptide as claimed in anyone of claims 1 to 3 and/or a polynucleotide as claimed in claim 4 or 5 and/or an antibody as claimed in anyone of claims 11 to 13.

Abstract

The present invention relates to a 40.9 kDa allergen from mugwort pollen, to polypeptides derived therefrom and polynucleotides encoding the same. The invention further pertains to antibodies directed against the allergen and to the use of the materials described for the manufacture of a medicament for the treatment or prevention of an allergic disorder.

Figure 1A

1	M4cDNA	GG AATTCGGCAC GAG---AAAT CTACAAAAAT TGATAAAAAAT	80
	M6cDNA	GG AATTCGGCAC GAG---AA-T CTACAAAAAT TGATAAAAAAT	
	M8cDNA	GGGCGCGCT CTAGAACTAG TGGATCCCCC GGGCTGCAGG AATTCGGCAC GAG-----AAAT TGATAAAAAAT	
	M15cDNA	GGGCGCGCT CTAGAACTAG TGGATCCCCC GGGCTGCAGG AATTCGGCAC GAGTGAATAT CTACAAAAAT TGATAAAAAAT	
	Consensus	
81	M4cDNA	AAAAATAA-- ----CAAAGC GAGTCATTGG CTTGCATACA TGGCTACATG ATTGCGCTTTA GACAACACAA TAAATAATCA	160
	M6cDNA	AAAAATAAAA ATAACAAAGC GAGTCATTGG CTTGCATACA TGGCTACATG ATTGCGCTTTA GACAACACAA TAAATAATCA	
	M8cDNA	AAAAATAAAA ATAACAAAGC GAGTCATTGG CTTGCATACA TGGCTACATG ATTGCGCTTTA GACAACACAA TAAATAATCA	
	M15cDNA	AAAAATAAAA ATAACAAAGC GAGTCATTGG CTTGCATACA TGGCTACATG ATTGCGCTTTA GACAACACAA TAAATAATCA	
	Consensus	AAAAATAaaa ataaCAAAGC GAGTCATTGG CTTGCATACA TGGCTACATG ATTGCGCTTTA GACAACACAA TAAATAATCA	
161	M4cDNA	GCAATATATA AAGTACCTTC GGTACTTTGA GATAGAAAGT TGTAAAAAAA GATAATCATA CAATACAATG GAAAAACATT	240
	M6cDNA	GCAATATATA AAGTACCTTC GGTACTTTGA GATAGAAAGT TGTAAAAAAA GATAATCATA CAATACAATG GAAAAACATT	
	M8cDNA	GCAATATATA AAGTACCTTC GGTACTTTGA GATAGAAAGT TGTAAAAAAA GATAATCATA CAATACAATG GAAAAACATT	
	M15cDNA	GCAATATATA AAGTACCTTC GGTACTTTGA GATAGAAAGT TGTAAAAAAA GATAATCATA CAATACAATG GAAAAACATT	
	Consensus	GCAATATATA AAGTACCTTC GGTACTTTGA GATAGAAAGT TGTAAAAAAA GATAATCATA CAATACAATG GAAAAACATT	
241	M4cDNA	ATTTTGTAT ATTGTTTACC GCAGCGTTTG TTTTCGTGGG TGCAGCTGCT CGGGCTGACA TTGGTGATGA GCTCGAAGCG	320
	M6cDNA	ATTTTGTAT ATTGTTTACC GCAGCGTTTG TTTTCGTGGG TGCAGCTGCT CGGGCTGACA TTGGTGATGA GCTCGAAGCG	
	M8cDNA	ATTTTGTAT ATTGTTTACC GCAGCGTTTG TTTTCGTGGG TGCAGCTGCT CGGGCTGACA TTGGTGATGA GCTCGAAGCG	
	M15cDNA	ATTTTGTAT ATTGTTTACC GCAGCGTTTG TTTTCGTGGG TGCAGCTGCT CGGGCTGACA TTGGTGATGA GCTCGAAGCG	
	Consensus	ATTTTGTAT ATTGTTTACC GCAGCGTTTG TTTTCGTGGG TGCAGCTGCT CGGGCTGACA TTGGTGATGA GCTCGAAGCG	
321	M4cDNA	GCTCAATTTA ATTCAACAAG GAGGGGCTTA CACGAATGTG CAGCATATAA CATAATAGAC AAGTGTGGA GGTGCAAAGC	400
	M6cDNA	GCTCAATTTA ATTCAACAAG GAGGGGCTTA CACGAATGTG CAGCATATAA CATAATAGAC AAGTGTGGA GGTGCAAAGC	
	M8cDNA	GCTCAATTTA ATTCAACAAG GAGGGGCTTA CACGAATGTG CAGCATATAA CATAATAGAC AAGTGTGGA GGTGCAAAGC	
	M15cDNA	GCTCAATTTA ATTCAACAAG GAGGGGCTTA CACGAATGTG CAGCATATAA CATAATAGAC AAGTGTGGA GGTGCAAAGC	
	Consensus	GCTCAATTTA ATTCAACAAG GAGGGGCTTA CACGAATGTG CAGCATATAA CATAATAGAC AAGTGTGGA GGTGCAAAGC	

	401	TGATTGGGAA	AAAAACCGAC	AAGCATTAGC	CAAAATGCCG	CAAGGTTTTG	CAAAGGGAAC	AACTGGCGGA	TTGGGAGGGG	481
M4cDNA		TGATTGGGAA	AAAAACCGAC	AAGCATTAGC	CAAAATGCCG	CAAGGTTTTG	CAAAGGGAAC	AACTGGCGGA	TTGGGAGGGG	
M6cDNA		TGATTGGGAA	AAAAACCGAC	AAGCATTAGC	CAAAATGCCG	CAAGGTTTTG	CAAAGGGAAC	AACTGGCGGA	TTGGGAGGGG	
M8cDNA		TGATTGGGAA	AAAAACCGAC	AAGCATTAGC	CAAAATGCCG	CAAGGTTTTG	CAAAGGGAAC	AACTGGCGGA	TTGGGAGGGG	
M15cDNA		TGATTGGGAA	AAAAACCGAC	AAGCATTAGC	CAAAATGCCG	CAAGGTTTTG	CAAAGGGAAC	AACTGGCGGA	TTGGGAGGGG	
consensus		TGATTGGGAA	AAAAACCGAC	AAGCATTAGC	CAAAATGCCG	CAAGGTTTTG	CAAAGGGAAC	AACTGGCGGA	TTGGGAGGGG	
	481	AAATCTACGT	GGTGACTGAT	TGTTTCAGATG	ACAATGCTGC	AAATCCAAAG	CCAGGGACAC	TTCGTTGTGG	TGTCACCCCAA	561
M4cDNA		AAATCTACGT	GGTGACTGAT	TGTTTCAGATG	ACAATGCTGC	AAATCCAAAG	CCAGGGACAC	TTCGTTGTGG	TGTCACCCCAA	
M6cDNA		AAATCTACGT	GGTGACTGAT	TGTTTCAGATG	ACAATGCTGC	AAATCCAAAG	CCAGGGACAC	TTCGTTGTGG	TGTCACCCCAA	
M8cDNA		AAATCTACGT	GGTGACTGAT	TGTTTCAGATG	ACAATGCTGC	AAATCCAAAG	CCAGGGACAC	TTCGTTGTGG	TGTCACCCCAA	
M15cDNA		AAATCTACGT	GGTGACTGAT	TGTTTCAGATG	ACAATGCTGC	AAATCCAAAG	CCAGGGACAC	TTCGTTGTGG	TGTCACCCCAA	
consensus		AAATCTACGT	GGTGACTGAT	TGTTTCAGATG	ACAATGCTGC	AAATCCAAAG	CCAGGGACAC	TTCGTTGTGG	TGTCACCCCAA	
	561	GATAAACCTT	TGTGGATCAT	CTTTAAGAAA	GATATGGTCA	TAAAACTTAA	ACACGAGCTT	GTGATAAACA	AAGACAAGAC	641
M4cDNA		GATAAACCTT	TGTGGATCAT	CTTTAAGAAA	GATATGGTCA	TAAAACTTAA	ACACGAGCTT	GTGATAAACA	AAGACAAGAC	
M6cDNA		GATAAACCTT	TGTGGATCAT	CTTTAAGAAA	GATATGGTCA	TAAAACTTAA	ACACGAGCTT	GTGATAAACA	AAGACAAGAC	
M8cDNA		GATAAACCTT	TGTGGATCAT	CTTTAAGAAA	GATATGGTCA	TAAAACTTAA	ACACGAGCTT	GTGATAAACA	AAGACAAGAC	
M15cDNA		GATAAACCTT	TGTGGATCAT	CTTTAAGAAA	GATATGGTCA	TAAAACTTAA	ACACGAGCTT	GTGATAAACA	AAGACAAGAC	
consensus		GATAAACCTT	TGTGGATCAT	CTTTAAGAAA	GATATGGTCA	TAAAACTTAA	ACACGAGCTT	GTGATAAACA	AAGACAAGAC	
	641	AATTGATGGA	AGAGGTGCAA	ATGTTGAGAT	CAC TTGTGGC	GGTCTCACCA	TTCAACAACGT	TTGCAATGTG	ATCATTCATA	721
M4cDNA		AATTGATGGA	AGAGGTGCAA	ATGTTGAGAT	CAC TTGTGGC	GGTCTCACCA	TTCAACAACGT	TTGCAATGTG	ATCATTCATA	
M6cDNA		AATTGATGGA	AGAGGTGCAA	ATGTTGAGAT	CAC TTGTGGC	GGTCTCACCA	TTCAACAACGT	TTGCAATGTG	ATCATTCATA	
M8cDNA		AATTGATGGA	AGAGGTGCAA	ATGTTGAGAT	CAC TTGTGGC	GGTCTCACCA	TTCAACAACGT	TTGCAATGTG	ATCATTCATA	
M15cDNA		AATTGATGGA	AGAGGTGCAA	ATGTTGAGAT	CAC TTGTGGC	GGTCTCACCA	TTCAACAACGT	TTGCAATGTG	ATCATTCATA	
consensus		AATTGATGGA	AGAGGTGCAA	ATGTTGAGAT	CAC TTGTGGC	GGTCTCACCA	TTCAACAACGT	TTGCAATGTG	ATCATTCATA	
	721	ACATTCACAT	ACATGATATT	AAAGTAACCG	AAGGTGGAAT	TATTAAAGGCA	ACGGACCGCTA	AACCAGGACA	TAGACATAAG	801
M4cDNA		ACATTCACAT	ACATGATATT	AAAGTAACCG	AAGGTGGAAT	TATTAAAGGCA	ACGGACCGCTA	AACCAGGACA	TAGACATAAG	
M6cDNA		ACATTCACAT	ACATGATATT	AAAGTAACCG	AAGGTGGAAT	TATTAAAGGCA	ACGGACCGCTA	AACCAGGACA	TAGACATAAG	
M8cDNA		ACATTCACAT	ACATGATATT	AAAGTAACCG	AAGGTGGAAT	TATTAAAGGCA	ACGGACCGCTA	AACCAGGACA	TAGACATAAG	
M15cDNA		ACATTCACAT	ACATGATATT	AAAGTAACCG	AAGGTGGAAT	TATTAAAGGCA	ACGGACCGCTA	AACCAGGACA	TAGACATAAG	
consensus		ACATTCACAT	ACATGATATT	AAAGTAACCG	AAGGTGGAAT	TATTAAAGGCA	ACGGACCGCTA	AACCAGGACA	TAGACATAAG	

File 1D

	1280	
	1281	
	1282	
	1283	
	1284	
	1285	
	1286	
	1287	
	1288	
	1289	
	1290	
	1291	
	1292	
	1293	
	1294	
	1295	
	1296	
	1297	
	1298	
	1299	
	1300	
	1301	
	1302	
	1303	
	1304	
	1305	
	1306	
	1307	
	1308	
	1309	
	1310	
	1311	
	1312	
	1313	
	1314	
	1315	
	1316	
	1317	
	1318	
	1319	
	1320	
	1321	
	1322	
	1323	
	1324	
	1325	
	1326	
	1327	
	1328	
	1329	
	1330	
	1331	
	1332	
	1333	
	1334	
	1335	
	1336	
	1337	
	1338	
	1339	
	1340	
	1341	
	1342	
	1343	
	1344	
	1345	
	1346	
	1347	
	1348	
	1349	
	1350	
	1351	
	1352	
	1353	
	1354	
	1355	
	1356	
	1357	
	1358	
	1359	
	1360	
	1361	
	1362	
	1363	
	1364	
	1365	
	1366	
	1367	
	1368	
	1369	
	1370	
	1371	
	1372	
	1373	
	1374	
	1375	
	1376	
	1377	
	1378	
	1379	
	1380	
	1381	
	1382	
	1383	
	1384	
	1385	
	1386	
	1387	
	1388	
	1389	
	1390	
	1391	
	1392	
	1393	
	1394	
	1395	
	1396	
	1397	
	1398	
	1399	
	1400	
	1401	
	1402	
	1403	
	1404	
	1405	
	1406	
	1407	
	1408	
	1409	
	1410	
	1411	
	1412	
	1413	
	1414	
	1415	
	1416	
	1417	
	1418	
	1419	
	1420	
	1421	
	1422	
	1423	
	1424	
	1425	
	1426	
	1427	
	1428	
	1429	
	1430	
	1431	
	1432	
	1433	
	1434	
	1435	
	1436	
	1437	
	1438	
	1439	
	1440	
	1441	
	1442	
	1443	
	1444	
	1445	
	1446	
	1447	
	1448	
	1449	
	1450	
	1451	
	1452	
	1453	
	1454	
	1455	
	1456	
	1457	
	1458	
	1459	
	1460	
	1461	
	1462	
	1463	
	1464	
	1465	
	1466	
	1467	
	1468	
	1469	
	1470	
	1471	
	1472	
	1473	
	147	

Figure 2

cDNA and amino acid sequences of M4, M6, M15 (identical clones):

```

atggaaaaacattatTTTTgttatattgttcaccgcagcgTTTTgttttcgtgggtgcagct
M E K H Y F V I L F T A A F V F V G A A
gctcgggctgacattgggtgatgagctcgaagcggctcaatttaattcaacaaggaggggc
A R A D I G D E L E A A Q F N S T R R G
ttacacgaatgtgcagcacataacataatagacaagtgttgagggtgcaaagctgattgg
L H E C A A H N I I D K C W R C K A D W
gaaaaaaaccgacaagcattagccaaatgcgcgcaaggTTTTgcaaagggaacaactggc
E K N R Q A L A K C A Q G F A K G T T G
ggattgggaggggaaatctacgtggtgactgattgttcagatgacaatgctgcaaatcca
G L G G E I Y V V T D C S D D N A A N P
aagccagggacacttcgttgtggtgtcacccaagataaacctttgtggatcatctttaag
K P G T L R C G V T Q D K P L W I I F K
aaagatatggtcataaaaacttaaacacgagcttgtgataaacaagacaagacaattgat
K D M V I K L K H E L V I N K D K T I D
ggaagaggtgcaaattgttgagatcacttgtggcggtctcaccattcacaacgtttgcaat
G R G A N V E I T C G G L T I H N V C N
gtgatcattcataacattcacatacatgatattaaagtaaccgaaggtggaattattaag
V I I H N I H I H D I K V T E G G I I K
gcaacggacgctaaaccaggacatagacataagagcgacggagatggtatTTgtgttgct
A T D A K P G H R H K S D G D G I C V A
ggttcttcaaagatatggatcgatcattgcacacttagtcatgggtccagatggccttatt
G S S K I W I D H C T L S H G P D G L I
gatgtcacgttgggtagcacagccggttaccattttccaattgcaaatttagccatcaccaa
D V T L G S T A V T I S N C K F S H H Q
aaaattctattactcggagcagacaattcacatgtagacgataaaaaaatgcatgtcaca
K I L L L G A D N S H V D D K K M H V T
gtagcattcaacaggttcgcagaagcatgtgatcaaagaatgccacgatgtcgatttgga
V A F N R F A E A C D Q R M P R C R F G
TTTTccaagttgttaacaatgactacaccagctggggaacgtacgccattggtggtagt
F F Q V V N N D Y T S W G T Y A I G G S
gccaatcctactatccttagccaaggcaaccgattccatgctccgaatgaccaatgaag
A N P T I L S Q G N R F H A P N D P M K
aaaaatgtgttggtgagggctgatgcaccacatacagagtcaatgaagtggaattggaga
K N V L V R A D A P H T E S M K W N W R
tctgagaaagacttgttagaaaatggagctatatTTgttagcatcaggggtgcgacccgcac
S E K D L L E N G A I F V A S G C D P H
ctaaccccggaacaaaaaagccattttgattccagctgaaccaggatcagcagttcttcaa
L T P E Q K S H L I P A E P G S A V L Q
ctcaccagttgtgctggcacgctcaaatgcgttcctggaaaaccttgttaa
L T S C A G T L K C V P G K P C -

```

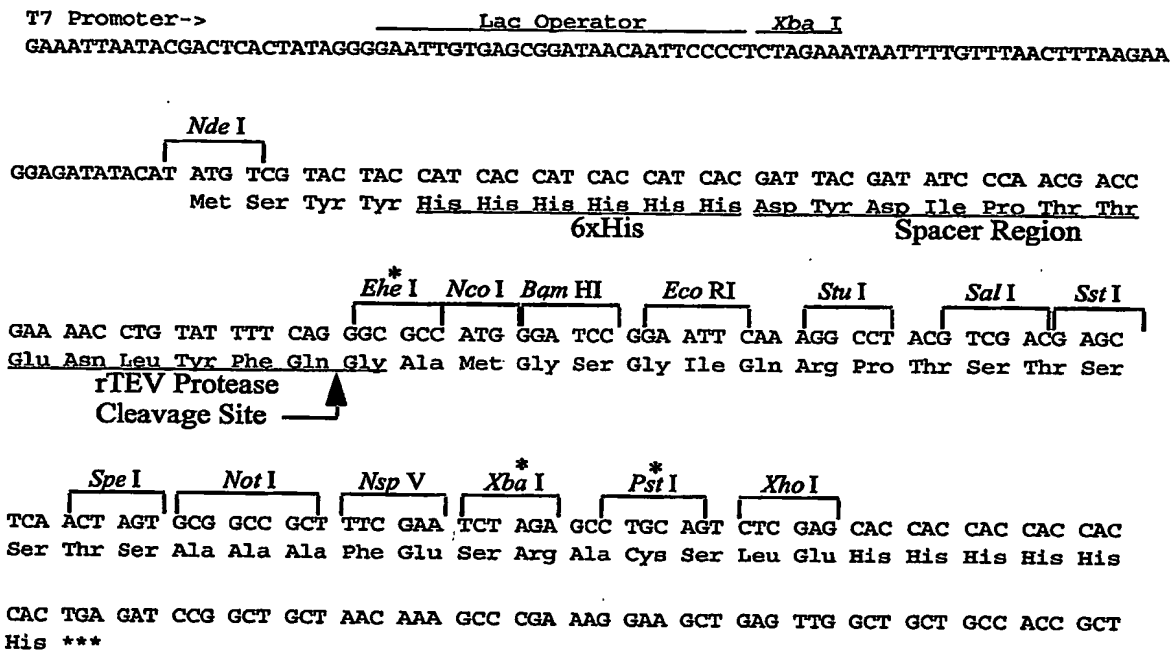
Figure 3**cDNA and amino acid sequence of clone M8:**

```

atggaaaaacattatTTTTgttatattgttcaccgcagcgtttgttttcgtgggtgcagct
M E K H Y F V I L F T A A F V F V G A A
gctcgggctgacattggtgatgagctcgaagcggctcaatttaattcaacaaggaggggc
A R A D I G D E L E A A Q F N S T R R G
ttacacgaatgtgcagcacataacataatagacaagtgttgagggtgcaaagctgattgg
L H E C A A H N I I D K C W R C K A D W
gaaaaaaaccgacaagcattagccaaatgcgcgcaagggttttgcaaagggaacaactggc
E K N R Q A L A K C A Q G F A K G T T G
ggattgggaggggaaatctacgtggtgactgattgttcagatgacaatgctgcaaatcca
G L G G E I Y V V T D C S D D N A A N P
aagccagggacacttcgttgtggtgtcacccaagataaacctttgtggatcatcttcaag
K P G T L R C G V T Q D K P L W I I F K
aaagatatggtcataaaaacttaaacacgagcttgtgataaacaagacaagacaattgat
K D M V I K L K H E L V I N K D K T I D
ggaagaggtgcaaattgttgagatcacttgtggcgggtctcaccattcacaacgtttgcaat
G R G A N V E I T C G G L T I H N V C N
gtgatcattcataacattcacatacatgatattaaagtaacggaagggtggaattattaag
V I I H N I H I H D I K V T E G G I I K
gcaacggacgctaaccaggggcatagacataagagcgacggagatggtatTTTgtgttgct
A T D A K P G H R H K S D G D G I C V A
ggttcttcgaagatatggatcgatcattgcacacttagtcatggtccagatggccttatt
G S S K I W I D H C T L S H G P D G L I
gatgtcacggttggttagcacagccgttaccatttccaattgcaaatttagccatcaccaa
D V T L G S T A V T I S N C K F S H H Q
aaaattctattactcggagcagacaattcacatgtagacgataaaaaaatgcatgtcaca
K I L L L G A D N S H V D D K K M H V T
gtcgcattcaacagggttcgcagaagcatgtgatcaaagaatgccacgatgtcgatttgga
V A F N R F A E A C D Q R M P R C R F G
tttttccaagttgttaacaatgactacaccagctggggaacgtacgccatttggtggtagc
F F Q V V N N D Y T S W G T Y A I G G S
gccaatcctactatccttagccaaggcaaccgattccatgctcccaatgacccaatgaag
A N P T I L S Q G N R F H A P N D P M K
aaaaatgtgttggtgagggctgatgcaccacatacagagtcaatgaagtggaattggaga
K N V L V R A D A P H T E S M K W N W R
tctgagaaagacttgttagaaaaatggagctatatTTTtagcatcagggtgcgacccgcat
S E K D L L E N G A I F V A S G C D P H
ctaaccccggaacaaaaaagccattttaattccagctgaaccaggatcagcagttcttcaa
L T P E Q K S H L I P A E P G S A V L Q
ctcaccagttgtgctggcagcgtcaaatgcgttccttgaaaaaccttgtaa
L T S C A G T L K C V P G K P C -

```

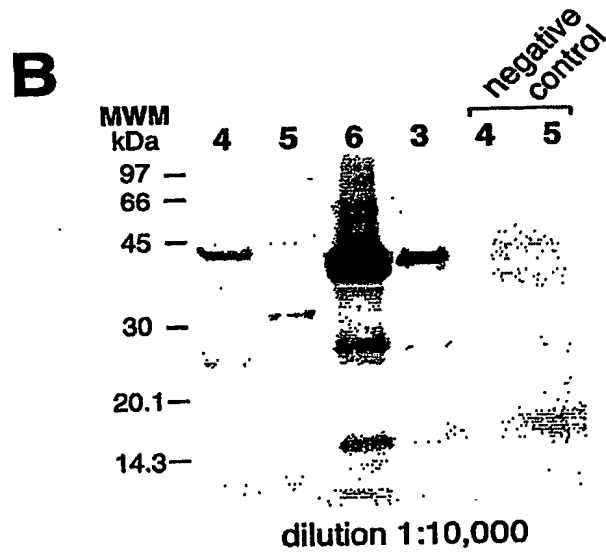
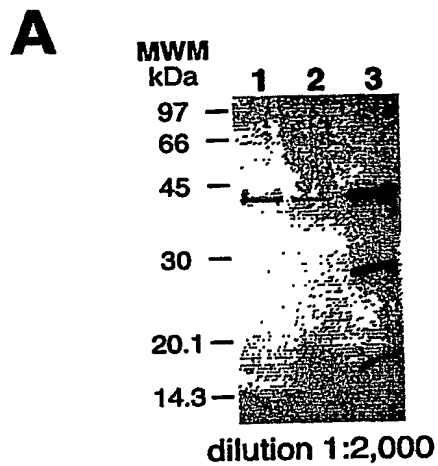
Figure 4

pHIS-Parallel2

*Non-unique sites

Immunoblot with rabbit anti-Amb a 1 antibodies

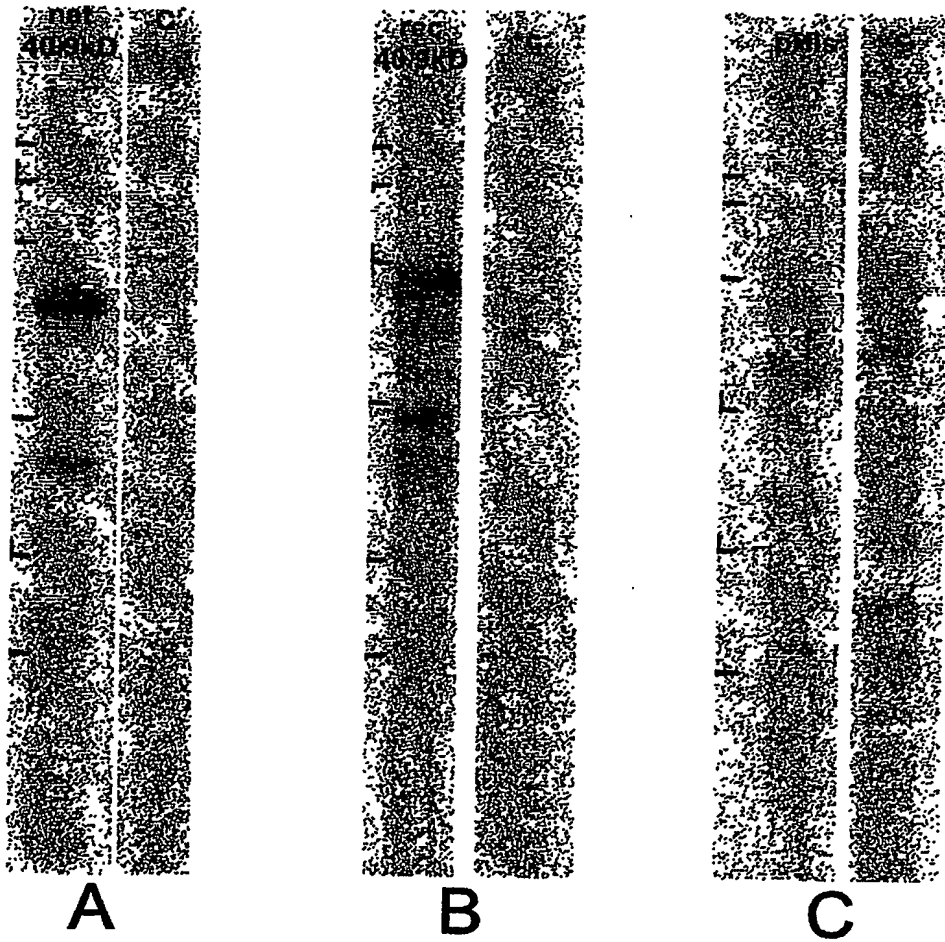
(Figure 5)



- 1 - Mugwort pollen extract
- 2 - Purified mugwort pollen allergen
- 3 - Purified Amb a 1 from ragweed pollen (natural Amb a 1)
- 4 - Recombinant mugwort allergen
- 5 - Control bacterial lysate
- 6 - Ragweed pollen extract

Figure 6

IgE blot with NIH patient



SEQUENCE LISTING

<110> Biomay Produktions- und Handels-Aktiengesellschaft

<120> Allergenic protein from mugwort pollen

<130> Allergenic protein from mugwort pollen

<140>

<141>

<160> 9

<170> PatentIn Ver. 2.1

<210> 1

<211> 396

<212> PRT

<213> Artemisia vulgaris

<400> 1

Met Glu Lys His Tyr Phe Val Ile Leu Phe Thr Ala Ala Phe Val Phe
1 5 10 15

Val Gly Ala Ala Ala Arg Ala Asp Ile Gly Asp Glu Leu Glu Ala Ala
20 25 30

Gln Phe Asn Ser Thr Arg Arg Gly Leu His Glu Cys Ala Ala His Asn
35 40 45

Ile Ile Asp Lys Cys Trp Arg Cys Lys Ala Asp Trp Glu Lys Asn Arg
50 55 60

Gln Ala Leu Ala Lys Cys Ala Gln Gly Phe Ala Lys Gly Thr Thr Gly
65 70 75 80

Gly Leu Gly Gly Glu Ile Tyr Val Val Thr Asp Cys Ser Asp Asp Asn
85 90 95

Ala Ala Asn Pro Lys Pro Gly Thr Leu Arg Cys Gly Val Thr Gln Asp
100 105 110

Lys Pro Leu Trp Ile Ile Phe Lys Lys Asp Met Val Ile Lys Leu Lys
115 120 125

His Glu Leu Val Ile Asn Lys Asp Lys Thr Ile Asp Gly Arg Gly Ala
130 135 140

Asn Val Glu Ile Thr Cys Gly Gly Leu Thr Ile His Asn Val Cys Asn
145 150 155 160

Val Ile Ile His Asn Ile His Ile His Asp Ile Lys Val Thr Glu Gly
165 170 175

Gly Ile Ile Lys Ala Thr Asp Ala Lys Pro Gly His Arg His Lys Ser
180 185 190

Asp Gly Asp Gly Ile Cys Val Ala Gly Ser Ser Lys Ile Trp Ile Asp
195 200 205

His Cys Thr Leu Ser His Gly Pro Asp Gly Leu Ile Asp Val Thr Leu
210 215 220

Gly Ser Thr Ala Val Thr Ile Ser Asn Cys Lys Phe Ser His His Gln
225 230 235 240

Lys Ile Leu Leu Leu Gly Ala Asp Asn Ser His Val Asp Asp Lys Lys
245 250 255

Met His Val Thr Val Ala Phe Asn Arg Phe Ala Glu Ala Cys Asp Gln
260 265 270

Arg Met Pro Arg Cys Arg Phe Gly Phe Phe Gln Val Val Asn Asn Asp
275 280 285

Tyr Thr Ser Trp Gly Thr Tyr Ala Ile Gly Gly Ser Ala Asn Pro Thr
290 295 300

Ile Leu Ser Gln Gly Asn Arg Phe His Ala Pro Asn Asp Pro Met Lys
305 310 315 320

Lys Asn Val Leu Val Arg Ala Asp Ala Pro His Thr Glu Ser Met Lys
325 330 335

Trp Asn Trp Arg Ser Glu Lys Asp Leu Leu Glu Asn Gly Ala Ile Phe
340 345 350

Val Ala Ser Gly Cys Asp Pro His Leu Thr Pro Glu Gln Lys Ser His
355 360 365

Leu Ile Pro Ala Glu Pro Gly Ser Ala Val Leu Gln Leu Thr Ser Cys
370 375 380

Ala Gly Thr Leu Lys Cys Val Pro Gly Lys Pro Cys
385 390 395

<210> 2

<211> 1188

<212> DNA

<213> *Artemisia vulgaris*

<400> 2

```
atggaaaaac attatattgt tatattgttc accgcagcgt ttgttttcgt ggggtgcagct 60
gctcgggctg acattggtga tgagctcgaa gcggctcaat ttaattcaac aaggaggggc 120
ttacacgaat gtgcagcaca taacataata gacaagtgtt ggaggtgcaa agctgattgg 180
gaaaaaaacc gacaagcatt agccaaatgc gcgcaagggt ttgcaaaggg aacaactggc 240
ggattgggag gggaaatcta cgtggtgact gattgttcag atgacaatgc tgcaaattcca 300
aagccaggga cacttcggtg tgggtgcacc caagataaac ctttgtggat catctttaag 360
aaagatatgg tcataaaact taaacacgag cttgtgataa acaaagacaa gacaattgat 420
ggaagagggt caaatgttga gatcacttgt ggcgggtctca ccattcacia cgtttgcaat 480
gtgatcattc ataacattca catacatgat attaaagtaa ccgaagggtg aattattaag 540
gcaacggacg ctaaaccagg acatagacat aagagcgacg gagatgggtat ttgtgttgct 600
ggttcttcaa agatatggat cgatcattgc acacttagtc atggtccaga tggccttatt 660
gatgtcacgt tgggtagcac agccgttacc atttccaatt gcaaatttag ccatcaccia 720
aaaattctat tactcggagc agacaattca catgtagacg ataaaaaaat gcatgtcaca 780
gtagcattca acagggttcg agaagcatgt gatcaaagaa tgccacgatg tcgatttgga 840
tttttccaag ttgttaacaa tgactacacc agctggggaa cgtacgccat tgggtgtagt 900
gccaatccta ctatccttag ccaaggcaac cgattccatg ctccgaatga cccaatgaag 960
aaaaatgtgt tgggtagggc tgatgcacca catacagagt caatgaagtg gaattggaga 1020
tctgagaaag acttgttaga aaatggagct atattttag catcagggtg cgacccgcac 1080
ctaaccgccg aacaaaaaag ccatttgatt ccagctgaac caggatcagc agttcttcaa 1140
ctcaccagtt gtgctggcac gtc aaatgc gttcctggaa aaccttgt 1188
```

<210> 3

<211> 1188

<212> DNA

<213> *Artemisia vulgaris*

<400> 3

```
atggaaaaac attatattgt tatattgttc accgcagcgt ttgttttcgt ggggtgcagct 60
gctcgggctg acattggtga tgagctcgaa gcggctcaat ttaattcaac aaggaggggc 120
ttacacgaat gtgcagcaca taacataata gacaagtgtt ggaggtgcaa agctgattgg 180
gaaaaaaacc gacaagcatt agccaaatgc gcgcaagggt ttgcaaaggg aacaactggc 240
ggattgggag gggaaatcta cgtggtgact gattgttcag atgacaatgc tgcaaattcca 300
aagccaggga cacttcggtg tgggtgcacc caagataaac ctttgtggat catcttcaag 360
aaagatatgg tcataaaact taaacacgag cttgtgataa acaaagacaa gacaattgat 420
ggaagagggt caaatgttga gatcacttgt ggcgggtctca ccattcacia cgtttgcaat 480
gtgatcattc ataacattca catacatgat attaaagtaa ccgaagggtg aattattaag 540
gcaacggacg ctaaaccagg gcatagacat aagagcgacg gagatgggtat ttgtgttgct 600
ggttcttcca agatatggat cgatcattgc acacttagtc atggtccaga tggccttatt 660
gatgtcacgt tgggtagcac agccgttacc atttccaatt gcaaatttag ccatcaccia 720
aaaattctat tactcggagc agacaattca catgtagacg ataaaaaaat gcatgtcaca 780
```

gtgcattca acaggttcgc agaagcatgt gatcaaagaa tgccacgatg tgcatttgga 840
 tttttccaag ttgttaacaa tgactacacc agctggggaa cgtacgccat tgggtggtagc 900
 gccaatccta ctatccttag ccaaggcaac cgattccatg ctcccaatga cccaatgaag 960
 aaaaatgtgt tgggtgagggc tgatgcacca catacagagt caatgaagtg gaattggaga 1020
 tctgagaaag acttggttaga aaatggagct atattttag catcaggggtg cgacccgcac 1080
 ctaaccccg aacaaaaaag ccatttaatt ccagctgaac caggatcagc agttcttcaa 1140
 ctcaccagtt gtgctggcac gctcaaatgc gttcctggaa aaccttgt 1188

<210> 4

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 4

gagagagacc atggctcggg ctgacattgg tgatgagctc g

41

<210> 5

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 5

gagagagact cgagttaaca aggttttcca ggaacgcatt tg

42

<210> 6

<211> 1523

<212> DNA

<213> Artemisia vulgaris

<400> 6

ggaattcggc acgagaaatc tacaaaaatt gataaaaaata aaaataacaa agcgagtcac 60
 tggcttgcac acatggctac atgattcgct ttagacaaca caataaataa tcagcaatat 120
 ataaagtacc ttccgtactt tgagatagaa agttgtaaaa aaagataatc atacaatata 180
 atggaaaaac attattttgt tatattgttc accgcagcgt ttgttttcgt ggggtgcagct 240
 gctcgggctg acattggtga tgagctcgaa gcggctcaat ttaattcaac aaggaggggc 300
 ttacacgaat gtgcagcaca taacataata gacaagtgtt ggaggtgcaa agctyattgg 360
 gaaagaaatc gacaagcatt agccaaatgc ggcgaagggt ttgcaaaggg aacaactggc 420
 gaaagaaatc gacaagcatt agccaaatgc ggcgaagggt ttgcaaaggg aacaactggc 480
 gaaagaaatc gacaagcatt agccaaatgc ggcgaagggt ttgcaaaggg aacaactggc 540

aaagatatgg	tcataaaact	taaacacgag	cttgtgataa	acaaagacaa	gacaattgat	600
ggaagagggtg	caaattgttg	gatcacttgt	ggcgggtctca	ccattcacaa	cgtttgcaat	660
gtgatcattc	ataacattca	catacatgat	attaaagtaa	ccgaagggtg	aattattaag	720
gcaacggacg	ctaaaccagg	acatagacat	aagagcgacg	gagatggat	ttgtgttgct	780
ggttcttcaa	agatatggat	cgatcattgc	acacttagtc	atgggtccaga	tggccttatt	840
gatgtcacgt	tgggtagcac	agccgttacc	atttccaatt	gcaaatttag	ccatcaccaa	900
aaaattctat	tactcggagc	agaacaattca	catgtagacg	ataaaaaaat	gcatgtcaca	960
gtagcattca	acaggttcgc	agaagcatgt	gatcaaagaa	tgccacgatg	tcgatttggg	1020
tttttccaag	ttgttaacaa	tgactacacc	agctggggaa	cgtaacgcat	tgggtggtagt	1080
gccaatccta	ctatccttag	ccaaggcaac	cgattccatg	ctccgaatga	cccaatgaag	1140
aaaaatgtgt	tgggtgagggc	tgatgcacca	catacagagt	caatgaagtg	gaattggaga	1200
tctgagaaag	acttgttaga	aaatggagct	atattttag	catcagggtg	cgacccgcat	1260
ctaaccctcg	aacaaaaaag	ccatttgatt	ccagctgaac	caggatcagc	agttcttcaa	1320
ctcaccagtt	gtgctggcac	gctcaaatgc	gttcctggaa	aaccttggtt	atagttatca	1380
cccacttttt	ttttttattg	ttatttagcat	tttttatact	tgttaggatt	gtagtggaa	1440
gagacattga	tacgtgcatt	acaagaccat	tcacaaacat	attttgctac	tatcacatgt	1500
tcacgtttaa	aaaaaaaaaa	aaa				1523

<210> 7

<211> 1513

<212> DNA

<213> *Artemisia vulgaris*

<400> 7

ggaattcggc	acgagaatct	acaaaaattg	ataaaaaataa	aaataaaaaat	aacaaagcga	60
gtcattgggt	tgcatacatg	gctacatgat	tcgctttaga	caacacaata	aataatcagc	120
aatatataaa	gtaccttcgg	tacttttgaga	tagaaagttg	taaaaaaaga	taatcataca	180
atacaatgga	aaaacattat	tttgttatat	tggtccaccg	agcgtttgtt	ttcgtgggtg	240
cagctgctcg	ggctgacatt	ggatgatgag	tcgaagcggc	tcaatttaat	tcaacaagga	300
ggggcttaca	cgaatgtgca	gcacataaca	taatagacaa	gtgttgagg	tgcaaaagctg	360
attgggaaaa	aaaccgacaa	gcatttagcca	aatgcgcgca	aggttttgca	aagggaacaa	420
ctggcgggatt	gggaggggaa	atctacgtgg	tgactgattg	ttcagatgac	aatgctgcaa	480
atccaaagcc	agggacactt	cgttgtggtg	tcaccaaga	taaacctttg	tggatcatct	540
ttaagaaaga	tatggtcata	aaacttaaac	acgagcttgt	gataaaca	gacaagacaa	600
ttgatggaag	aggtgcaaat	gttgagatca	cttgtggcgg	tctcaccatt	cacaacgttt	660
gcaatgtgat	cattcataac	attcacatac	atgatattaa	agtaaccgaa	gggtggaatta	720
ttaaggcaac	ggacgctaaa	ccaggacata	gacataagag	cgacggagat	ggatatttgtg	780
ttgctgggtc	ttcaaagata	tggatcgatc	attgcacact	tagtcatggt	ccagatggcc	840
ttattgatgt	cacgttgggt	agcacagccg	ttaccatttc	caattgcaaa	tttagccatc	900
acaaaaaat	tctattactc	ggagcagaca	attcacatgt	agacgataaa	aaaatgcatg	960
tcacagtagc	attcaacagg	ttcgcagaag	catgtgatca	aagaatgcc	cgatgtcgat	1020
ttggattttt	ccaagttgtt	aacaatgact	acaccagctg	gggaacgtac	gccattggtg	1080
gtagtgccaa	tctactatc	cttagccaag	gcaaccgatt	ccatgctccg	aatgacccaa	1140
tgaagaaaaa	tgtgttggtg	agggctgatg	caccacatac	agagtcaatg	aagtggaa	1200
ggagatctga	gaaagacttg	ttagaaaatg	gagctatatt	tgtagcatca	gggtgcgacc	1260
cgcatctaac	cccgaacaa	aaaagccatt	tgattccagc	tgaaccagga	tcagcagttc	1320
ttcaactcac	cagttgtgct	ggcacgctca	aatgcgttcc	tggaaaacct	tgtaaatagt	1380

tatcacccac tttttatattt tattgttatt agcatttttt atacttggtta ggattgtagt 1440
 ggaatgagac attgatacgt gcattacaag accatttcac aacatatattt gctaaaaaaa 1500
 aaaaaaaaaaaa aaa 1513

<210> 8

<211> 1520

<212> DNA

<213> Artemisia vulgaris

<400> 8

gcggccgctc tagaactagt ggatcccccg ggctgcagga attcggcacg agaaattgat 60
 aaaaaataaaa ataaaaataa caaagcgagt cattggcttg catacatggc tacatgattc 120
 gcttttagaca acacaataaa taatcagcaa tatataaagt accttcggta ctttgagata 180
 gaaagttgta aaaaaagata atcatacaat acaatggaaa aacattattt tgttatattg 240
 ttcaccgcag cgtttgtttt cgtgggtgca gctgctcggg ctgacattgg tgatgagctc 300
 gaagcggctc aattttaattc aacaaggagg ggcttacacg aatgtgcagc acataacata 360
 atagacaagt gttggagggtg caaagctgat tgggaaaaaa accgacaagc attagccaaa 420
 tgcgcgcaag gttttgcaaa gggaacaact ggcggtattg gaggggaaat ctacgtggtg 480
 actgattgtt cagatgacaa tgctgcaaat ccaaagccag ggacacttcg ttgtggtgtc 540
 acccaagata aacctttgtg gatcatcttc aagaaagata tggtcataaa acttaaacac 600
 gagcttgtga taaacaaaga caagacaatt gatggaagag gtgcaaattg tgagatcact 660
 tgtggcggctc tcaccattca caacgtttgc aatgtgatca ttcataacat tcacatacat 720
 gatattaaag taacggaagg tggaattatt aaggcaacgg acgctaaacc agggcataga 780
 cataagagcg acggagatgg tatttgtgtt gctggttctt cgaagatatg gatcgatcat 840
 tgcacactta gtcattggtc agatggcctt attgatgtca cgttgggtag cacagccgtt 900
 accatttcca attgcaaatt tagccatcac caaaaaattc tattactcgg agcagacaat 960
 tcacatgtag acgataaaaa aatgcatgtc acagtcgcat tcaacagggt cgcagaagca 1020
 tgtgatcaaa gaatgccacg atgtcgattt ggatttttcc aagttgttaa caatgactac 1080
 accagctggg gaacgtacgc cattggtggt agcgccaatc ctactatcct tagccaaggc 1140
 aaccgattcc atgctcccaa tgaccaatg aagaaaaatg tgttggtgag ggctgatgca 1200
 ccacatacag agtcaatgaa gtggaattgg agatctgaga aagacttgtt agaaaatgga 1260
 gctatatattg tagcatcagg gtgcgacccg catctaacc cggacaacaaa aagccattta 1320
 attccagctg aaccaggatc agcagttctt caactcacca gttgtgctgg cacgctcaaa 1380
 tgcgttcctg gaaaaccttg ttaatagtta tcaccaactt tttattttta ttgttattag 1440
 cattttttat acttgttagg attgtagtgg aatgagacat tgatacgtgc attacaagac 1500
 caaaaaaaaaa aaaaaaaaaa 1520

<210> 9

<211> 1535

<212> DNA

<213> Artemisia vulgaris

<400> 9

gcggccgctc tagaactagt ggatcccccg ggctgcagga aattcggcac gagtgaaaat 60
 aaaaaataaaa ataaaaataa caaagcgagt cattggcttg catacatggc tacatgattc 120
 gcttttagaca acacaataaa taatcagcaa tatataaagt accttcggta ctttgagata 180
 gaaagttgta aaaaaagata atcatacaat acaatggaaa aacattattt tgttatattg 240
 ttcaccgcag cgtttgtttt cgtgggtgca gctgctcggg ctgacattgg tgatgagctc 300
 gaagcggctc aattttaattc aacaaggagg ggcttacacg aatgtgcagc acataacata 360
 atagacaagt gttggagggtg caaagctgat tgggaaaaaa accgacaagc attagccaaa 420
 tgcgcgcaag gttttgcaaa gggaacaact ggcggtattg gaggggaaat ctacgtggtg 480
 actgattgtt cagatgacaa tgctgcaaat ccaaagccag ggacacttcg ttgtggtgtc 540
 acccaagata aacctttgtg gatcatcttc aagaaagata tggtcataaa acttaaacac 600
 gagcttgtga taaacaaaga caagacaatt gatggaagag gtgcaaattg tgagatcact 660
 tgtggcggctc tcaccattca caacgtttgc aatgtgatca ttcataacat tcacatacat 720
 gatattaaag taacggaagg tggaattatt aaggcaacgg acgctaaacc agggcataga 780
 cataagagcg acggagatgg tatttgtgtt gctggttctt cgaagatatg gatcgatcat 840
 tgcacactta gtcattggtc agatggcctt attgatgtca cgttgggtag cacagccgtt 900
 accatttcca attgcaaatt tagccatcac caaaaaattc tattactcgg agcagacaat 960
 tcacatgtag acgataaaaa aatgcatgtc acagtcgcat tcaacagggt cgcagaagca 1020
 tgtgatcaaa gaatgccacg atgtcgattt ggatttttcc aagttgttaa caatgactac 1080
 accagctggg gaacgtacgc cattggtggt agcgccaatc ctactatcct tagccaaggc 1140
 aaccgattcc atgctcccaa tgaccaatg aagaaaaatg tgttggtgag ggctgatgca 1200
 ccacatacag agtcaatgaa gtggaattgg agatctgaga aagacttgtt agaaaatgga 1260
 gctatatattg tagcatcagg gtgcgacccg catctaacc cggacaacaaa aagccattta 1320
 attccagctg aaccaggatc agcagttctt caactcacca gttgtgctgg cacgctcaaa 1380
 tgcgttcctg gaaaaccttg ttaatagtta tcaccaactt tttattttta ttgttattag 1440
 cattttttat acttgttagg attgtagtgg aatgagacat tgatacgtgc attacaagac 1500
 caaaaaaaaaa aaaaaaaaaa 1520

ggtactttga	gatagaaagt	tgtaaaaaaa	gataatcata	caatacaatg	gaaaaacatt	240
at tt t t g t t a t	a t t g t t c a c c	g c a g c g t t t g	t t t t c g t g g g	t g c a g c t g c t	c g g g c t g a c a	300
t t g g t g a t g a	g c t c g a a g c g	g c t c a a t t t a	a t t c a a c a a g	g a g g g g c t t a	c a c g a a t g t g	360
c a g c a c a t a a	c a t a a t a g a c	a a g t g t t g g a	g g t g c a a a g c	t g a t t g g g a a	a a a a a c c g a c	420
a a g c a t t a g c	c a a a t g c g c g	c a a g g t t t t g	c a a a g g g a a c	a a c t g g c g g a	t t g g g a g g g g	480
a a a t c t a c g t	g g t g a c t g a t	t g t t c a g a t g	a c a a t g c t g c	a a a t c c a a a g	c c a g g g a c a c	540
t t c g t t g t g g	t g t c a c c c a a	g a t a a a c c t t	t g t g g a t c a t	c t t t a a g a a a	g a t a t g g t c a	600
t a a a a c t t a a	a c a c g a g c t t	g t g a t a a a c a	a a g a c a a g a c	a a t t g a t g g a	a g a g g t g c a a	660
a t g t t g a g a t	c a c t t g t g g c	g g t c t c a c c a	t t c a c a a c g t	t t g c a a t g t g	a t c a t t c a t a	720
a c a t t c a c a t	a c a t g a t a t t	a a a g t a a c c g	a a g g t g g a a t	t a t t a a g g c a	a c g g a c g c t a	780
a a c c a g g a c a	t a g a c a t a a g	a g c g a c g g a g	a t g g t a t t t g	t g t t g c t g g t	t c t t c a a a g a	840
t a t g g a t c g a	t c a t t g c a c a	c t t a g t c a t g	g t c c a g a t g g	c c t t a t t g a t	g t c a c g t t g g	900
g t a g c a c a g c	c g t t a c c a t t	t c c a a t t g c a	a a t t t a g c c a	t c a c c a a a a a	a t t c t a t t a c	960
t c g g a g c a g a	c a a t t c a c a t	g t a g a c g a t a	a a a a a a t g c a	t g t c a c a g t a	g c a t t c a a c a	1020
g g t t c g c a g a	a g c a t g t g a t	c a a a g a a t g c	c a c g a t g t c g	a t t t g g a t t t	t t c c a a g t t g	1080
t t a a c a a t g a	c t a c a c c a g c	t g g g g a a c g t	a c g c c a t t g g	t g g t a g t g c c	a a t c c t a c t a	1140
t c c t t a g c c a	a g g c a a c c g a	t t c c a t g c t c	c g a a t g a c c c	a a t g a a g a a a	a a t g t g t t g g	1200
t g a g g g c t g a	t g c a c c a c a t	a c a g a g t c a a	t g a a g t g g a a	t t g g a g a t c t	g a g a a a g a c t	1260
t g t t a g a a a a	t g g a g c t a t a	t t t g t a g c a t	c a g g g t g c g a	c c c g c a t c t a	a c c c c g g a a c	1320
a a a a a a g c c a	t t t g a t t c c a	g c t g a a c c a g	g a t c a g c a g t	t c t t c a a c t c	a c c a g t t g t g	1380
c t g g c a c g c t	c a a a t g c g t t	c c t g g a a a a c	c t t g t t a a t a	g t t a t c a c c c	a c t t t t t a t t	1440
t t t a t t g t t a	t t a g c a t t t t	t t a t a c t t g t	t a g g a t t g t a	g t g g a a t g a g	a c a t t g a t a c	1500
g t g c a t t a c a	a g a c c a a a a a	a a a a a a a a a a	a a a a a			1535

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.